Distribution, Metabolism, and Excretion of DDT and Mirex by a Marine Teleost, the Winter Flounder

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Vast quantities of highly persistent organochlorine compounds are released into the environment each year by man's agricultural and industrial activities *(1,2)*. Numerous examples of the severe consequences of intense, acute exposure to such agents have been documented *(3,4)*. At present, however, the larger problem is not one of intense contamination and short-term lethal effects; rather, it is of universal exposure to levels not yet overtly toxic. This is of primary importance in marine systems, particularly the highly productive coastal and estuarine waters, which are major recipients of these persistent compounds *(5,6)*.

As an initial step in examining such sublethal effects, we have looked at the fate and distribution of two organochlorine insecticides, DDT** and mirex in the winter flounder, *Pseudopleuronectes americanus.* DDT, of course, has been widely used, while mirex *(7,8)*, and its derivatives, e.g., Kepone *(9)*, are newer and have been used primarily for fire ant control in the southeastern United States. DDT and mirex share many properties, including thermal stability, resistance to photooxidation, high lipid solubility, and extreme persistence in the environment *(10—12)*. However, in structure, they are rather different. DDT (I) is a planar molecule containing a pair of aromatic rings. Mirex (II) is approximately cubic in configuration and is completely saturated with chlorine atoms. As will be shown below, they are alike in their later distribution, their excretion, and their lack of metabolism. On the other hand, they are quite different in their initial distribution and mobility within the flounder.

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**Chemical names of the compounds used in this paper are as follows: DDT = 1, 1, 1-trichloro-2, 2-bis(*p*-chlorophenyl)ethane; DDD - 1, 1-dichloro-2, 2-bis(*p*-chlorophenyl)ethane; DDE = 1, 1-dichloro-2, 2-bis(*p*-chlorophenyl)ethylene; DDA = 2, 2-bis(*p*-chlorophenyl)acetic acid; Kelthane - 1, 1, 1-trichloro-2, 2-bis(*p*-chlorophenyl)ethanol; DBP = 4. 4'-dichlorobenzophenone; Mirex = dodecachloro-octahydro-1, 3, 4-metheno-2H-cyclobuto[cd] pentalene; Kepone = dodecachlorooctahydro-1, 3, 4-metheno- 2H - cyclobuto [cd] pentalen -2-one; and DDMU = 1-chloro-2,2-bis(*p*-chlorophenyl)-ethylene.
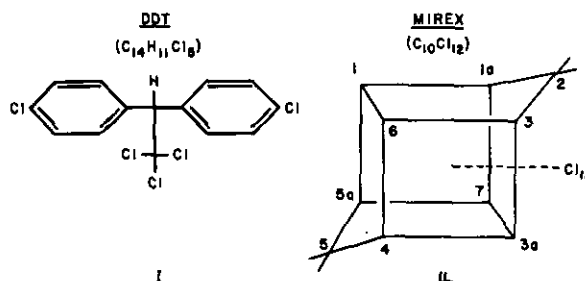

FIGURE 1. Chemical formulas and structures of DDT and mirex.

Methods and Materials

Fish

Winter flounder of 100-400 g were captured off Mount Desert Island, Maine by a small commercial dragger. Fish were maintained in large fiberglass tanks continuously flushed with running sea water and were fed fresh clam twice weekly.

Chemicals

Most unlabeled pesticides and derivatives were obtained in high purity from Aldrich Chemical Company. Kelthane was the gift of Rohm and Haas, Inc., and Kepone was extracted from a commercial preparation (TAT, Ant Trap, O. E. Linck Company, Clifton, New Jersey). Ring-labeled ^{14}C-p,p'-DDT was purchased from Amersham-Searle Corporation (specific activity = 23.9 μCi/μmole). The ^{14}C-mirex (specific activity 2.9 μCi/μmole) was obtained from Mallinckrodt, Inc.

Injection and Sampling

Fish were injected via the caudal vein with 100 μg/kg labeled pesticide dissolved in 50% ethanol and saline. Those fish to be maintained 48 hr or less were kept in enameled metal pans containing one liter of aerated sea water at 12-14°C. Fish maintained for 1 week were placed in free-flowing aquaria at a similar temperature. Blood samples were drawn by venipuncture of the caudal vein. Urine samples were collected via a catheter inserted into the external urinary papilla and sutured in place. Bile and tissue samples were obtained after decapitation of the fish. Carcass samples were obtained by homogenizing all reamining tissue, mostly muscle with skin and bone, after removal of other identifiable organs. Samples to be analyzed by liquid scintillation spectrometry were dissolved in 1 ml of Soluene (Packard Instrument Co.) and counted with 10 ml of toluene scintillation fluid (5 g PPO and 250 mg POPOP/l).

The enameled pans containing the fish were extracted with acetone, and an aliquot was analyzed by liquid scintillation counting as above. Finally, samples of the bathing sea water were analyzed by extracting an aliquot 1:1 with the toluene scintillation fluid and counting 10 ml of this extract.

Additional samples were extracted for thin layer chromatographic (TLC) analysis of pesticide metabolites. Tissues were homogenized for 2 min with 3 ml acetonitrile per gram tissue and sufficient perchloric acid to bring the mixture to pH 2, a modification of the technique of Fredeen et al. (13). The homogenate was centrifuged at 2000 g for 5 min. The resulting supernatant was concentrated under a stream of air. Fluid samples (plasma, bile, and urine) were similarly acidified, but were extracted three times with 2 ml heptane/ml fluid. Extracts were pooled and concentrated as above.

Thin-Layer Chromatography

Extracts were chromatographed in two or more of the following solvent systems: (1) heptane; (2) hexane: chloroform: acetic acid: ethyl ether (180:10:5:5); (3) heptane: ethanol (5:1); (4) hexane: acetic acid: ethyl ether (100:1:1); and (5) chloroform: acetone (9:1). Silica gel sheets (100 μ thick, Eastman Kodak Company) were used with all systems. Extracts were cochromatographed with authentic samples of DDT, DDD, DDE, DDA, DBP, Kelthane, mirex, and Kepone. The positions of the pesticides were determined by spraying with alcoholic $AgNO_3$ (0.5%) and exposure to ultraviolet light. The darkened regions of the plates containing the known standards were removed and counted, or the entire plate was cut into 1 or 0.5 cm strips which were counted individually employing the same scintillation fluid as above. R_{DDT} values obtained under these conditions for the standard compounds are reported in Table 1. In addition, two-dimensional chromatography in solvents 1 and 2 (above) provided more complete assurance of the identities of certain polar metabolites (Fig. 1).

Lipid Analysis

Tissue samples were extracted with chloroform-methanol (2:1), washed with Folch up-

per phase, i.e., chloroform−methanol−0.04% aqueous $CaCl_2$ (3:48:47), taken to dryness, and weighed according to the method of Folch *(14)*.

Table 1. R_f values of DDT analogs after thin-layer chromatography

	R_f[a]			
	Solvent 1	Solvent 2	Solvent 3	Solvent 4
DDA	0.00	0.30	0.15	0.08
DBP	0.08	0.84	1.00	0.75
KEL[b]	0.10	0.56	0.69	0.34
DDD	0.63	0.86	0.92	0.76
DDT	1.00	1.00	1.00	1.00
DDE	1.34	1.08	1.03	1.16

[a] R_f's expressed as R_{DDT}, where R_f of DDT is normalized to 1.00. Solvent 1, heptane; solvent 2, hexane − chloroform − acetic acid-ethyl ether (180:10:5:5); solvent 3, heptane−ethanol (5:1); solvent 4, hexane−acetic acid-ethyl ether (100:1:1).
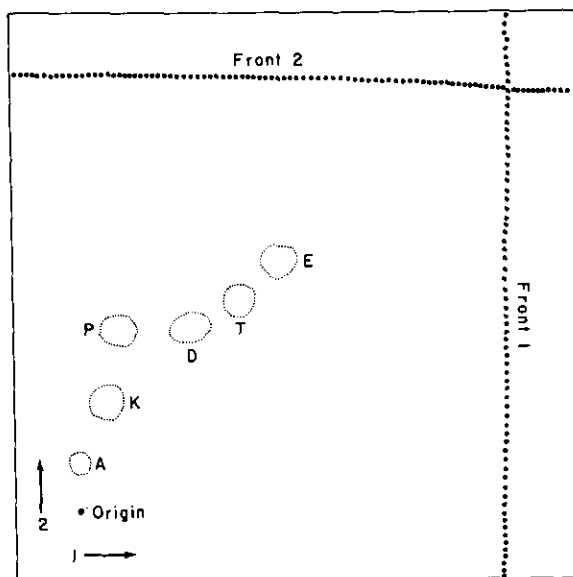
[b] Kelthane.

FIGURE 1. Reproduction of actual chromatograph showing typical separation of DDT and metabolites achieved by two-dimensional chromatography in solvents 1 and 2 (see text). A = DDA, K = Kelthane, P = DBP, D = DDD, T = DDT, and E = DDE.

Unlabeled Pesticide Analysis

Two flounder obtained from the same source as the experimental fish were sacrificed, immediately frozen, and less than 1 month later analyzed for DDT and metabolites by gas chromatography by using standardized procedures *(15)*. Briefly, whole flounder were ground in a blender to obtain a homogeneous sample and fat was extracted from a 20-ml aliquot. Following acetonitrile partition and Florisil chromatography, the extract was concentrated and analyzed on a Glowall Chromalab instrument equipped with a ^{226}Ra electron capture detector. The column was packed with a 1:1 mixture of 7% QF-1 and 9% OV-17 (both on 80/100 mesh Gas-Chrom Q) and run at 226°C. Identification and quantitation of sample peaks were performed by comparison of retention times and calibration curves obtained from standard solutions of reference compounds.

Results

Ambient Pesticide Level

Gas-chromatographic analysis of two freshly caught flounder provided an estimate of background DDT residues in the local flounder population of Mt. Desert Island, Maine (Table 2). On a wet weight basis, individual values for both fish were identical. Total DDT residues were 0.11 ppm in the whole body. Isomers of DDT itself (0.05 ppm) accounted for almost 50% of the pesticide, while DDD (0.02 ppm), DDE (0.01 ppm), and DDMU (0.03 ppm) made up the remainder. Since the lipid content of these wild flounder was so low (1.10 and 0.42 mg/g), expressing the data on a lipid weight basis must be viewed with caution. Under these conditions, lipid weight data may not only be misleading, but can be inaccurate as well *(16)*. Note that while values in the two fish were identical on a wet weight basis, they are grossly different on a lipid weight basis.

Total residues in these flounder approximate one fiftieth of the acute lethal dose we have observed in a preliminary experiment with the flounder. This value is in the lower

Table 2. Natural levels of DDT-related
compounds in the flounder[a]

| Compound | Amount found, ppm | |
	Wet weight	Fat weight
DDMU	0.03	43.1
		(29.8-62.4)
p,p'-DDE	0.01	20.6
		(11.4-29.8)
o,p'-DDD	0.01	4.94
		(2.74-7.14)
o,p'-DDT	0.02	25.7
		(14.7-36.7)
p,p'-DDD	0.01	6.59
		(3.65-9.52)
p,p'-DDT	0.03	50.1
		(29.2-71.0)
	Σ 0.11	151.03

[a]Mean value for two animals; numbers in parentheses
are ranges of values. Where no ranges are given,
replicate assays were equal. Assays were conducted
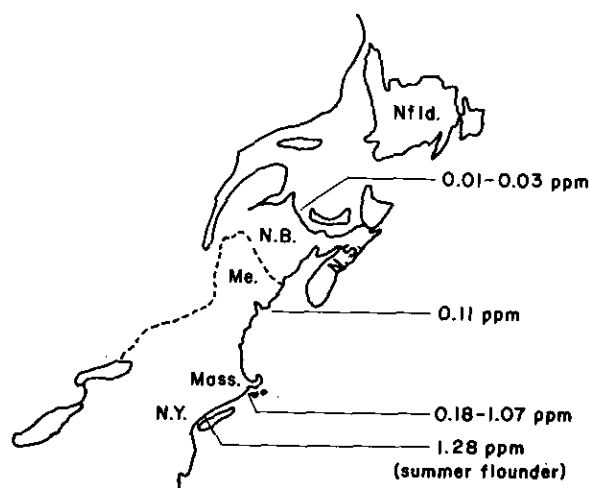as described in Methods.

FIGURE 2. DDT and metabolites in winter flounder
from northeastern United States and maritime
Canada expressed as parts per million on a wet
weight basis. Our value includes DDMU while the
other studies mentioned did not report this metab-
olite; therefore, a more comparable Maine value
might be 0.08 ppm.

portion of the spectrum of values thus far
reported for the winter flounder (Fig. 2).
Sprague and Duffy (17) reported values of
0.01-0.03 ppm off the coast of New Bruns-
wick. We found 0.11 ppm in Maine fish.
Smith and Cole (18) reported values up to
1.07 ppm in Massachusetts waters. Values in
another flatfish, the summer flounder, *Para-
lichthys dentatus*, taken in Long Island Sound
have been reported at 1.28 ppm (3). The
relatively low levels in Maine are in keeping
with shellfish monitoring data which demon-
strated lower contamination in Maine waters
than most other areas of coastal United States
(17).

Distribution of DDT and Mirex

The intravenous injections employed in this
study represented a dose of ^{14}C-pesticide
approximately equal to the existing environ-
mental load of 0.1 mg/kg of fish, i.e., 0.1
ppm. As will be shown below, metabolism of
both DDT and mirex is very limited, thus
measurement of total radioactivity is essen-
tially equivalent to the quantity of parent
pesticides. Table 3 shows the concentration of
^{14}C-pesticide in plasma at intervals after
injection. When the ^{14}C-DDT data were
plotted semilogarithmically, three compo-
nents were distinguished. An initial half time

Table 3. Plasma levels of ^{14}C-DDT or ^{14}C-mirex.[a]

| | Plasma level, μg/ml | | | | | | | |
	5 min	15 min	1 hr	4 hr	8 hr	24 hr	48 hr	1 wk
DDT	2.48	0.93	0.28	0.16	0.10	0.08	0.07	0.05
±SE	0.38	0.32	0.04	0.02	0.02	0.02	0.02	0.03
Mirex	—	0.50	0.06	0.14	—	0.14	—	0.08
±SE	—	0.13	0.02	0.02	—	0.02	—	0.02

[a]Both pesticides administered IV (tail vein) at dose of 100 μg/kg. Each value is mean ±SE (standard error) for
four to six animals.

$T_{1/2}$ of 5 min apparently represents (see below) tissue uptake from the vascular compartment. A second component with a half time of 2.25 hr presumably arises from redistribution of the drug from tissues of high blood flow and/or low affinity to tissues whose capacity has not yet been saturated, e.g., due to lower blood flow or higher affinity. In a third phase, there is a much slower fall in plasma concentration ($T_{1/2}$ = 10 days) reflecting an even slower redistribution component and/or excretion.

Plasma concentration data for ^{14}C-mirex is also included in Table 3. Unlike the continuous fall seen with DDT, mirex levels were variable at early times. From 4 hr, however, plasma mirex falls only slightly more slowly than plasma DDT. There seem to be only two components in the mirex curve, a rapid loss from the vascular compartment, followed by a slow ($T_{1/2}$ = 8 days) redistribution phase.

Tissue Distribution

Consideration of the tissue distribution of injected pesticide gives further indication of the movements described above. At early times when plasma levels were highest, all tissues were labeled (Table 4 and Fig. 3A). In the case of ^{14}C-DDT the carcass, liver, kidney, and plasma contained the bulk of the drug at 15 min and 1 hr. During the remainder of the week, the carcass (89% of body weight and primarily muscle) increased its content to over 80% of the pesticide, while plasma, liver, and all other organs lost pesticide. More specifically, during the period from 15 min to 24 hr, the carcass gained about 40% of the pesticide, while the other organs and plasma lost a comparable amount. This is reflected in the second component of the plasma decay curve for DDT ($T_{1/2}$ = 2.25 hr) and represents movement of DDT into what appears to be its ultimate sink, the muscle. Frequent checks with pure samples of muscle and of skin established that these two tissues consistently paralleled each other (skin contained slightly higher levels of both DDT and mirex) and that carcass values closely reflected the level of its major component, muscle. In view of the inherent problem of getting total muscle weight free of skin and bone, only carcass values are considered here.

Examining DDT distribution data from the perspective of tissue to plasma ratios (T/P) gives an appreciation for the concentration in

Table 4. Tissue distribution of ^{14}C-DDT and ^{14}C-mirex.[a]

	DDT, % of total		Mirex, % of total	
	15 min	1 wk	15 min	1 wk
Carcass (muscle, skin)	36.1 ± 4.3	82.8 ± 5.0	28.5 ± 11.4	54.8 ± 3.3
Liver	22.5 ± 2.0	4.1 ± 0.9	0.6 ± 0.2	12.1 ± 3.9
Plasma	16.3 ± 1.6	1.1 ± 0.5	9.9 ± 2.4	1.8 ± 0.4
Kidney	14.0 ± 6.5	0.5 ± 0.2	58.8 ± 12.5	16.3 ± 3.8
Gut	5.2 ± 0.6	1.3 ± 0.7	0.2 ± 0.1	1.2 ± 0.1
Gill	2.7 ± 0.4	0.7 ± 0.3	1.1 ± 0.5	0.9 ± 0.1
Stomach	1.4 ± 0.3	0.7 ± 0.4	0.1 ± 0.05	1.3 ± 0.2
Heart	0.9 ± 0.05	0.1 ± 0.03	0.4 ± 0.1	0.2 ± 0.05
Spleen	0.7 ± 0.1	0.1 ± 0.03	0.1 ± 0.04	1.4 ± 1.0
Gonad	0.4 ± 0.1	0.1 ± 0.03	<0.05	6.6 ± 2.1[b]
Brain	0.2 ± 0.01	0.1 ± 0.1	<0.05	0.1 ± 0.0
Bile	<0.05	6.5 ± 1.8	<0.05	2.2 ± 0.2
Urine	<0.05	1.9 ± 0.4	<0.05	0.4 ± 0.2
Water	<0.05	<0.05	0.3 ± 0.2	<0.05

[a] Both pesticides administered IV (tail vein) at dose of 100 μg/kg. Values are mean ±SE (standard error) for four to six animals analyzed at indicated time points after injected and are expressed as percent of total recovered pesticide in organ or fluid. Total recoveries were 81 and 92% for DDT and mirex, respectively.

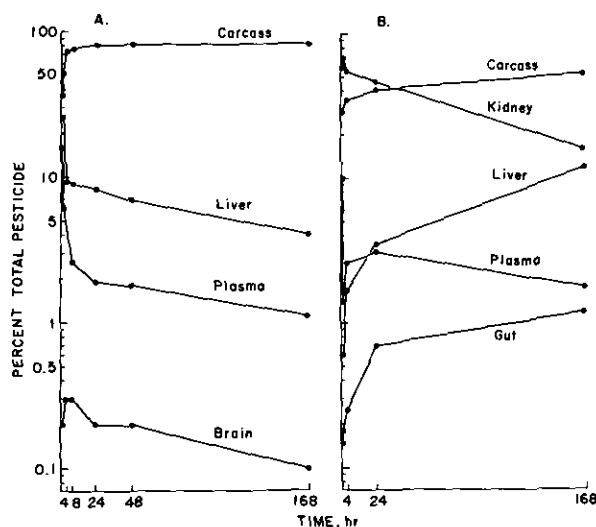[b] These fish were sexually ripe, therefore the gonads were very much larger.

FIGURE 3. Pesticide content of flounder tissues as per cent of total pesticide recovered vs. time after injection: *(A)* DDT; *(B)* Mirex.

FIGURE 4. Concentration of pesticide in representative tissues expressed as tissue to plasma ratios: *(A)* DDT; *(B)* mirex.

the tissues, normalized for the changing plasma levels to which the tissues were exposed (Fig. 4A). Once tissues were presented with DDT all T/P ratios began to increase and peaked by 8 hr. Thereafter, the ratios remained rather constant, i.e., tissue concentrations fell in parallel with plasma concentration. The only exception to this generalization is muscle, which showed a steady increase in T/P for 24 hr and a slight increase thereafter. Thus, by virtue of its size (about 89% of body weight), the carcass component acted as a sink and gradually accumulated the bulk of the pesticide even though the actual pesticide concentration did not become as great as that in several smaller organs such as liver (only 0.84% body weight).

The mirex distribution picture is comparable to that of DDT, except its time course is extended. The renal compartment was the dominant one initially (Table 4 and Fig. 3B), since the route of administration presented a major portion of the dose to the kidney first via the caudal vein and the renal portal system. Other modes of administration (stomach tube or uptake from ambient water) showed accumulation in the first organs exposed (i.e., gut and liver or gill). Thus, it was a
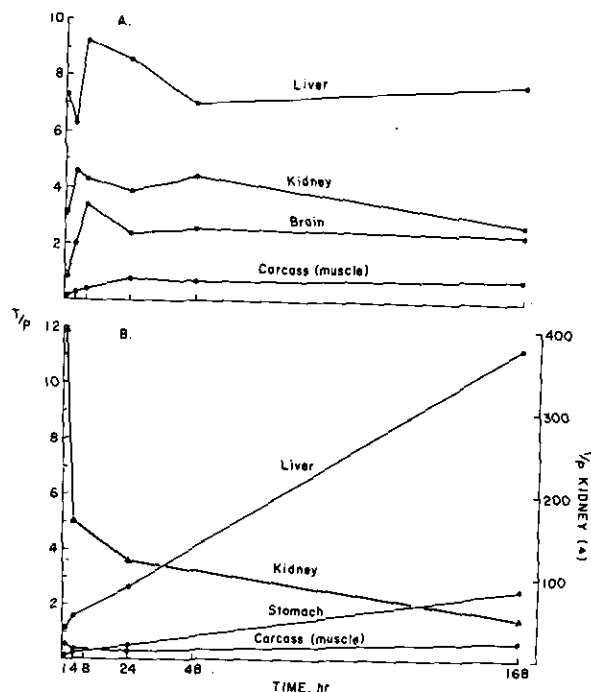
large initial exposure to, not a high renal affinity for, mirex which produced the apparently large "accumulation" of mirex in the kidney at early times. The 15-min data for DDT also showed an elevated renal value; however, the time course for redistribution was much more rapid compared with mirex. This redistribution of mirex from the renal compartment to other organs appears to be the major factor in the prolonged ($T_{1/2}$ = 8 days) component of the plasma decay curve. All other tissues increased in mirex content during this period (Fig. 3B), the quantitatively most significant recipients being liver and carcass. Similarly, all tissue except kidney required an extended period to achieve maximum T/P ratio for mirex, and in fact, all T/P ratios, except kidney, rose throughout the entire exposure period (Fig. 4B). The overall impression is that a distribution similar to DDT was being approached slowly. Longer-term exposure would, of course, be necessary to confirm this impression.

Basis of Distribution Pattern

Because of their extensive lipid solubility one might ask how the distribution patterns for DDT and mirex relate to the distribution of lipid in the flounder. Table 5 clearly demonstrates that there is no correlation ($r \leqslant$ 0.2) with lipid content at any time up to 1 week after exposure. The brain, which has the highest lipid concentrations, has the least DDT per milligram lipid. Gonad has the lowest lipid concentration in these sexually immature fish; nevertheless, it shows relatively high DDT per milligram lipid. One is forced to acknowledge that the total lipid concentration in the recipient tissue does not determine initial DDT uptake. Mirex is extreme in this regard, since injection via the caudal vein presents the drug initially to the kidney which accumulates the majority of the initial dose. On the other hand, at least with DDT, the slight increase in correlation coefficient during the week's exposure leaves open the possibility that the ultimate distribution may more closely reflect lipid distribution.

Excretion

Excretion of both pesticides was slow by all routes (Table 4 and Fig. 5). As determined by the almost complete absence of radioactivity in the bathing sea water and in the acetone extract from the walls of the container, net gill excretion was negligible (less than 0.1% of the dose in 1 week). Loss with reabsorption is, of course, possible (20). Urinary excretion was slight (1.9% of the dose in one week for DDT and 0.5% for mirex). Both of these observations are explained, at least in part, by our preliminary plasma binding studies of DDT, which demonstrated that 95% or more of the pesticide is bound and thus is not available for either diffusion at the gills or filtration by the kidney.

Bile appeared to be the major route of excretion for these pesticides, since 6.5% and 2.2% of DDT and mirex, respectively, was excreted in this manner in 1 week. However, this was determined in gall bladder bile; thus, the pesticide it contained had not transversed the digestive tract where it could have been reabsorbed. Several preliminary experiments

Table 5. Relationship of DDT distribution to flounder tissue lipid content.[a]

Tissue	Lipid, mg/g	^{14}C-DDT ng/mg lipid		
		1 hr	24 hr	1 wk
Brain	87.6	2.4	2.2	1.8
Liver	42.6	47.7	16.2	8.0
Kidney	28.2	30.5	11.0	4.3
Muscle	13.7	2.2	4.4	3.6
Gonad	8.6	25.6	10.5	8.1
Correlation coefficient (r)		0.001	0.063	0.197

[a]Values are mean weights ^{14}C-DDT/mg lipid/g wet weight for four to six fish at each time period. Since the lipid content of gonads increase (ca. 40 mg/g) in mature gonads, only sexually immature fish were employed in this segment of the study.
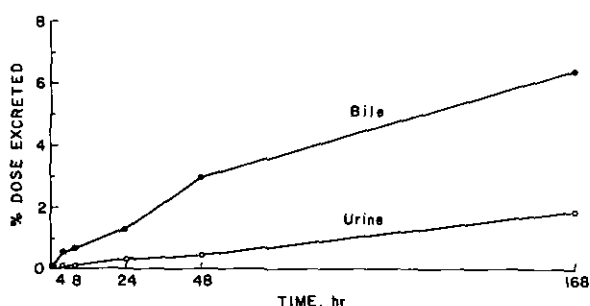

FIGURE 5. Biliary and renal excretion of ^{14}C-DDT (% dose excreted) vs. time after injection.

presenting each pesticide via stomach tube have shown that both DDT and mirex may be absorbed from the gut of the flounder. Thus, net biliary excretion must be even less than we have measured. The rate of excretion for DDT was relatively constant via both the renal and biliary routes (Fig. 5). Biliary excretion of mirex gave much the same picture. The rate of urinary excretion was not constant, since there was little additional urinary excretion after 4 hr (0.4% of the dose in 4 hr compared with 0.5% in 1 week). Thus, the bulk of both of the intravascularly injected pesticides, particularly mirex, is retained by the fish.

Metabolism

On extracting the samples and analyzing the extracts as described above, it was established that all tissues and fluids, except urine,

contained primarily unaltered ^{14}C-DDT or -mirex. Representative data for DDT in tissues and fluids are shown in Table 6. As exemplified by liver and plasma data, DDT itself generally accounted for more than 90% of the label at all times studied. Even bile conformed to this pattern. Considering all values from all tissues and fluids, except urine, DDT averaged 93.2% of the label, DDD 2.6%, DDE 1.3%, and taken together, polar metabolites 2.9%. Mirex gave no evidence of metabolism in any sample. Each chromatogram showed only a single species which cochromatographed with the parent compound, ^{14}C-mirex.

The only exception to the general paucity of metabolites in this study was in the urine of DDT-treated fish (Table 6). TLC in the heptane system showed that urine contained a preponderance of one or more polar metabolites (50-70%). The other major component was DDT (15-28%), with lesser amounts of DDE and DDD detected. Analysis of urine

Table 6. DDT and its metabolites in flounder tissues and body fluids.[a]

	[14]C, % of total			
	1 hr	4 hr	24 hr	1 wk
Liver[b]				
DDT	94.8	94.6	92.1	93.6
Polar	0.5	2.0	2.2	2.1
DDD	4.0	2.2	3.3	2.1
DDE	0.7	1.2	2.5	2.1
Plasma				
DDT	91.2	87.4	96.0	96.9
Polar	4.3	5.2	2.7	1.6
DDD	2.9	3.0	1.4	0.0
DDE	1.6	4.4	0.0	1.6
Urine				
DDT	—[c]	17.8	27.5	14.8
Polar	—	50.0	58.2	69.8
DDD	—	10.7	7.2	8.9
DDE	—	21.4	7.2	6.2

[a] Values are means for 2-4 fish at each time point after IV (tail vein) injection of 100 μg/kg of ^{14}C-DDT and are in terms of per cent total [14]C.

[b] Kidney, brain, gonad, and bile gave values similar to those of liver and plasma.

[c] Urinary values at 1 hr too low (< 100 cpm) to measure.

extracts in the other solvent systems, including two dimensional chromatography demonstrated that: (1) a compound cochromatographing with DDA in each of the systems accounted for 60-90% of the polar metabolites, and (2) a second component behaving similarly to dichlorobenzophenone (DBP) was also present. Kelthane was not detected in fish extracts. The polar component of liver, plasma, and bile extracts had comparable behavior upon two-dimensional chromatography, i.e., a compound cochromatographing with DDA predominated, and a smaller fraction possibly representing DBP was also present. This is, to our knowledge, the first description of the DDA metabolite in fish, although its presence in other vertebrates and even in one insect has been known for some time (12). Further evidence suggesting that the winter flounder is able to produce DDA is provided by the presence of DDMU in the flounder analyzed for background pesticide levels. DDMU has been shown to be an intermediate in the conversion of DDT to DDA by the rat (21). Clearly, however, the activity of the necessary enzymes is low. No tissue or fluid except urine contains more than a few per cent metabolites, and even urine accounts for less than 2% of the dose by 1 week.

Discussion

Upon reaching the bloodstream, both pesticides are rapidly taken up by the tissues. Despite their high lipid solubility, DDT and mirex are not initially distributed in accord with the lipid content of the recipient flounder tissues. Holden (22) demonstrated a similar lack of initial correlation between ^{14}C-DDT and lipid content in the brown trout (Salmo trutto) after uptake from water. By 1 week to 10 days, however, he found a much better correlation, although brain DDT content was still conspicuously low. A comparable result was obtained in the rat by Herd et al. (23), who demonstrated clearly that the initial (3 hr) distribution of ^{3}H-DDT was a function of blood flow while the later (3 wk) distribution was in accord with lipid content.

Thus, it is reasonable to picture the initial distribution of DDT or mirex as a rapid movement from the relatively aqueous environment of plasma to many sites of varying affinity in the tissues, and therefore dependent primarily upon relative perfusion of the sites. Furthermore, our own preliminary plasma-binding studies and those of others *(24)* argue that after the initial period essentially all pesticide remaining in plasma is also bound to some macromolecular site. After the initial distribution, there is redistribution from sites of low affinity to those of higher affinity (i.e., lipid). Thus, the toxic effects of an acute exposure may be very different from those of long-term, low-level dosage, since different organs of different sensitivities will be more heavily exposed, depending upon dose, time course, and route of exposure.

A further possibility for variation in toxic effects is provided by the different lipid contents among species. For example, Dvorchik and Maren *(24)* showed in the dogfish, *Squalus acanthias*, which has a large liver (10−17% of body weight) both heavily perfused and high in lipid (40−60% of wet weight), that this organ immediately takes up the bulk of the ^{14}C-DDT entering the fish. Moreover, the liver retains the pesticide and the dogfish does not show the redistribution characteristic of the *winter flounder*, which has a small liver (0.8% of body weight) relatively low in lipid (4% of wet weight). Thus, the liver of the dogfish is an effective sink for the pesticide and diminishes the exposure of other organs to a lower level than in the flounder.

In addition to distribution, a *second major factor* influencing the toxicity of these pesticides is the ability of the fish to metabolize them. In the flounder, impaired metabolism results in slow conversion of the parent pesticides to less toxic derivatives, i.e., over 90% of the DDT and all of the mirex present after 1 week is still the unaltered parent compound. Furthermore, limited metabolism *minimizes production of more water-soluble, more easily excreted products such as DDA.* It should be emphasized that while polar metabolites comprised only a few per cent of the total label present, they accounted for 40-80% of the urinary DDT levels. Since plasma binding *(24)* also minimizes the availability of the parent pesticide, excretion of both DDT and mirex is very slow. Thus, the winter flounder retains a large fraction of the pesticide presented to it typifying the concept of bioconcentration, and is essentially unable to reduce the toxicity of the retained pesticide. Hence, it would appear that many of the general concepts regarding the fate and distribution of DDT *(1−6)* and mirex *(25−28)* in the biosphere and in certain marine species can be successfully applied to the winter flounder. Exceptions to these general rules can be explained in terms of certain unique features this species possesses, e.g., relative organ size, relative fat content, blood vessel shunting, and limited ability to metabolize chlorinated hydrocarbon pesticides. One final consequence is also evident. Since the winter flounder holds its pesticide burden primarily in muscle, the flesh of flounder from contaminated areas will be heavily loaded and is potentially dangerous for terminal consumers, including man.

Acknowledgement

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